



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/40, 31/445, 31/535, C07D 209/12, 209/14, 401/10, 401/14, 413/10, 413/14	A1	(11) International Publication Number: WO 98/48797 (43) International Publication Date: 5 November 1998 (05.11.98)
(21) International Application Number: PCT/US98/08698 (22) International Filing Date: 30 April 1998 (30.04.98) (30) Priority Data: 60/045,136 30 April 1997 (30.04.97) US (71) Applicant (for all designated States except US): ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indi- anapolis, IN 46285 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CHIRGADZE, Nickolay Yuri [RU/US]; 14960 Beacon Boulevard, Carmel, IN 46032 (US). FISCHER, Matthew, Joseph [US/US]; 4106 Armon Court, Carmel, IN 46033 (US). HARPER, Richard, Waltz [US/US]; Apartment 806, 3025 North Meridian Street, Carmel, IN 46033 (US). LIN, Ho-Shen [US/US]; 8128 Trevellian Way, Indianapolis, IN 46217 (US). MCCOWAN, Jefferson, Ray [US/US]; 2653 Crescent Hill Lane, Indi- anapolis, IN 46208 (US). SALL, Daniel, Jon [US/US]; 376 Leisure Lane, Greenwood, IN 46142 (US). SMITH, Gerald, Floyd [US/US]; 825 Queenswood Court, Indianapolis, IN 46217 (US). TAKEUCHI, Kumiko [US/US]; 6342 Robin- srock Drive, Indianapolis, IN 46268 (US). WILEY, Michael,		Robert [US/US]; 7725 Langwood Drive, Indianapolis, IN 46268 (US). ZHANG, Minsheng [CN/US]; 31 Scheurman Terrace, Warren, NJ 07059 (US). (74) Agents: JACKSON, Thomas, E. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: ANTITHROMBOTIC AGENTS (57) Abstract This application relates to the use as thrombin inhibitors, coagulation inhibitors and thromboembolic disorder agents of heterocyclic derivatives of formula (I) as defined herein. It also provides novel compounds of formula (I), processes and intermediates for their preparation, and pharmaceutical formulations comprising the novel compounds of formula (I).		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

-1-

ANTITHROMBOTIC AGENTS

5 This invention relates to thrombin inhibitors
which are useful anticoagulants in mammals. In particular,
it relates to heterocyclic derivatives having high
anticoagulant activity, and antithrombotic activity. Thus,
this invention relates to new inhibitors of thrombin,
10 pharmaceutical compositions containing the compounds as
active ingredients, and the use of the compounds as
anticoagulants for prophylaxis and treatment of
thromboembolic disorders such as venous thrombosis,
pulmonary embolism, arterial thrombosis, in particular
15 myocardial ischemia, myocardial infarction and cerebral
thrombosis, general hypercoagulable states and local
hypercoagulable states, such as following angioplasty and
coronary bypass operations, and generalized tissue injury as
it relates to the inflammatory process. In addition, the
20 antithrombotic agents are useful as anticoagulants in in
vitro applications.

 The process of blood coagulation, thrombosis, is
triggered by a complex proteolytic cascade leading to the
formation of thrombin. Thrombin proteolytically removes
25 activation peptides from the A α -chains and the B β -chains of
fibrinogen, which is soluble in blood plasma, initiating
insoluble fibrin formation.

 Anticoagulation currently is achieved by the
administration of heparins and coumarins. Parenteral
30 pharmacological control of coagulation and thrombosis is
based on inhibition of thrombin through the use of heparins.

-2-

Heparins act indirectly on thrombin by accelerating the inhibitory effect of endogenous antithrombin III (the main physiological inhibitor of thrombin). Because antithrombin III levels vary in plasma and because clot-bound thrombin seems resistant to this indirect mechanism, heparins can be an ineffective treatment. Because coagulation assays are believed to be associated with efficacy and with safety, heparin levels must be monitored with coagulation assays (particularly the activated partial thromboplastin time (APTT) assay). Coumarins impede the generation of thrombin by blocking the posttranslational gamma-carboxylation in the synthesis of prothrombin and other proteins of this type. Because of their mechanism of action, the effect of coumarins can only develop slowly, 6-24 hours after administration. Further, they are not selective anticoagulants. Coumarins also require monitoring with coagulation assays (particularly the prothrombin time (PT) assay).

Recently, interest has grown in small synthetic molecules which demonstrate potent direct inhibition of thrombin. See, for example Robert M. Scarborough, Annual Reports in Medicinal Chemistry, (1995), 30, 71-80.

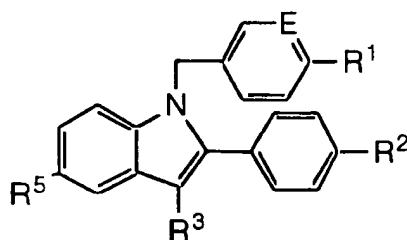
Although the heparins and coumarins are effective anticoagulants, no commercial drug has yet emerged from the small synthetic molecules; and despite the continuing promise for this class of compounds, there still exists a need for anticoagulants which act selectively on thrombin, and which, independent of antithrombin III, exert inhibitory action shortly after administration, preferably by an oral route, and do not interfere with lysis of blood clots, as required to maintain hemostasis.

The present invention is directed to the discovery that the compounds of the present invention, as defined below, are potent thrombin inhibitors that may have high bioavailability following oral administration.

According to the invention there is provided a method of inhibiting thrombin comprising using an effective

-3-

amount of a thrombin inhibiting compound of formula I (or a pharmaceutically acceptable salt thereof)



5

wherein

E is CH or CR^e in which R^e is methyl, methoxy or halo;

R¹ is carboxy, [(1-4C)alkoxy]carbonyl, hydroxymethyl or -X¹-(CH₂)_s-NR^sR^t in which X¹ is a direct bond, methylene or O; s is 1 or 2; provided that when s is 1, then X³ is a direct bond; and R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino;

R² is benzyloxy, -X²-(CH₂)_m-NR^aR^b in which X² is a direct bond, methylene, O or S; m is 1, 2, 3, 4 or 5; provided that when m is 1, then X² is a direct bond; and R^a and R^b are independently hydrogen or (1-3C)alkyl or the group NR^aR^b is pyrrolidino, piperidino, or morpholino; or

R² is -X²-(CH₂)_n-R^f in which X² is a direct bond, methylene or O; n is 1, 2 or 3; and R^f is 5-tetrazolyl, carboxy, [(1-4C)alkoxy]carbonyl or hydroxymethyl;

R³ is hydrogen, chloro or a benzyl group which may bear a methyl or methoxy substituent at the 3-position and a [(1-4C)alkoxy]carbonyl substituent at the 4-position; and

R⁵ is hydrogen, hydroxy or methoxy; and provided that at least one of R¹ and R² includes an amino moiety -NR^sR^t or -NR^aR^b.

Particularly, the compound of formula I is one in which

-4-

E is CH or CR^e in which R^e is methyl, methoxy or bromo;

R¹ is -X¹-(CH₂)_s-NR^sR^t;

5 R² is -X²-(CH₂)_m-NR^aR^b or is -X²-(CH₂)_n-R^f in which X² is O; n is 3; and R^f is carboxy, [(1-4C)alkoxy]-carbonyl or hydroxymethyl;

R³ is hydrogen or chloro; and

R⁵ is hydrogen, hydroxy or methoxy.

10 More particularly, the compound of formula I is one in which

E is CH or CR^e in which R^e is methoxy;

15 R¹ is -X¹-(CH₂)_s-NR^sR^t in which X¹ is a direct bond, methylene or O; s is 1 or 2; provided that when s is 1, then X³ is a direct bond; and R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino;

20 R² is -X²-(CH₂)_m-NR^aR^b in which X² is O, m is 2, and the group NR^aR^b is pyrrolidino, piperidino, or morpholino; or

R² is -X²-(CH₂)_n-R^f in which X² is O; n is 1, 2 or 3; and R^f is carboxy or [(1-4C)alkoxy]carbonyl;

R³ is hydrogen; and

R⁵ is hydrogen, hydroxy or methoxy.

25

Particular values for the groups include those in which, independently:

E is CH or CR^e in which R^e is methoxy.

R¹ is pyrrolidinomethyl or 2-pyrrolidinoethoxy.

30 R² is 2-pyrrolidinoethoxy or R² is -X²-(CH₂)_n-R^f in which X² is O, n is 3, and R^f is carboxy or methoxy-carbonyl;

R³ is hydrogen; and

R⁵ is hydrogen.

35

A preferred method of the invention includes one wherein said compound of formula I is the one described herein at Example 1.

-5-

The present invention also provides a method of inhibiting coagulation in a mammal comprising administering to a mammal in need of treatment, a coagulation inhibiting dose of a thrombin inhibiting compound of formula I having
5 any of the above definitions.

The present invention further provides a method of inhibiting thrombin comprising administering to a mammal in need of treatment, a thrombin inhibiting dose of a thrombin inhibiting compound of formula I having any of the above
10 definitions.

Further, the present invention provides a method of treating a thromboembolic disorder comprising administering to a mammal in need of treatment, an effective dose of a thrombin inhibiting compound of formula I having
15 any of the above definitions.

As an additional aspect of the invention there is provided a pharmaceutical formulation comprising in association with a pharmaceutically acceptable carrier, diluent or excipient, a thrombin inhibiting compound of
20 formula I (or a pharmaceutically acceptable salt thereof) as provided in any of the above descriptions.

In addition, there is provided the use of a thrombin inhibiting compound of formula I having any of the above definitions for the manufacture of a medicament for
25 treatment of a thromboembolic disorders.

As a further aspect of the invention, there is provided a prodrug (or a pharmaceutically acceptable salt thereof) of any of the above described thrombin inhibiting compounds of formula I which will form a prodrug. (It will
30 be recognized that a thrombin inhibiting compound of formula I also may serve as a prodrug for a different thrombin inhibiting compound of formula I).

As an additional feature of the invention there is provided a pharmaceutical formulation comprising in
35 association with a pharmaceutically acceptable carrier, diluent or excipient, a prodrug of a thrombin inhibiting

-6-

compound of formula I (or of a pharmaceutically acceptable salt thereof) as provided in any of the above descriptions.

In general, the thrombin inhibiting compounds of formula I are believed to be novel and, thus, to constitute an additional aspect of the invention. However, in European Patent Application, Publication Number 802183, with a publication date of 22 October 1997 (subsequent to the date from which this application claims priority), there may be generic disclosure of an intermediate for synthesis which is a benzylated phenolic (benzyloxyphenyl) compound corresponding to a compound of formula I wherein E is CH or CR^e in which R^e is methyl, methoxy or halo; R¹ is -X¹-(CH₂)_s-NR^sR^t in which X¹ is O; s is 2; R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino; R² is benzyloxy; R³ is hydrogen or chloro; and R⁵ is hydrogen, hydroxy or methoxy. Thus, according to the invention there is provided a novel compound of formula I (or a pharmaceutically acceptable salt thereof) according to any of the above definitions of a compound of formula I, provided that the compound is not one wherein E is CH or CR^e in which R^e is methyl, methoxy or halo; R¹ is -X¹-(CH₂)_s-NR^sR^t in which X¹ is O; s is 2; R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino; R² is benzyloxy; R³ is hydrogen or chloro; and R⁵ is hydrogen, hydroxy or methoxy.

A pharmaceutically acceptable salt of an antithrombotic agent of the instant invention includes one which is an acid-addition salt made with an acid which provides a pharmaceutically acceptable anion or a salt made with a base which provides a pharmaceutically acceptable cation. Thus, a pharmaceutically acceptable salt of a novel compound of formula I as provided above provides a particular aspect of the invention. Examples of such salts are provided hereinbelow.

As an additional aspect of the invention there is provided a pharmaceutical formulation comprising in

-7-

association with a pharmaceutically acceptable carrier, diluent or excipient, a novel compound of formula I (or a pharmaceutically acceptable salt thereof) as provided in any of the above descriptions.

5 In this specification, the following definitions are used, unless otherwise described: Halo is fluoro, chloro, bromo or iodo. Alkyl, alkoxy, etc. denote both straight and branched groups; but reference to an individual radical such as "propyl" embraces only the straight chain
10 ("normal") radical, a branched chain isomer such as "isopropyl" being specifically denoted.

 It will be appreciated that certain compounds of formula I (or salts or prodrugs, etc.) may exist in, and be isolated in, isomeric forms, including cis- or trans-
15 isomers, as well as optically active, racemic, or diastereomeric forms. It is to be understood that the present invention encompasses a compound of formula I as a mixture of diastereomers, as well as in the form of an individual diastereomer, and that the present invention
20 encompasses a compound of formula I as a mixture of enantiomers, as well as in the form of an individual enantiomer, any of which mixtures or form possesses inhibitory properties against thrombin, it being well known in the art how to prepare or isolate particular forms and
25 how to determine inhibitory properties against thrombin by standard tests including those described below.

 In addition, a compound of formula I (or salt or prodrug, etc.) may exhibit polymorphism or may form a solvate with water or an organic solvent. The present
30 invention also encompasses any such polymorphic form, any solvate or any mixture thereof.

 Particular values are listed below for radicals, substituents, and ranges, for illustration only, and they do not exclude other defined values or other values within
35 defined ranges for the radicals and substituents.

-8-

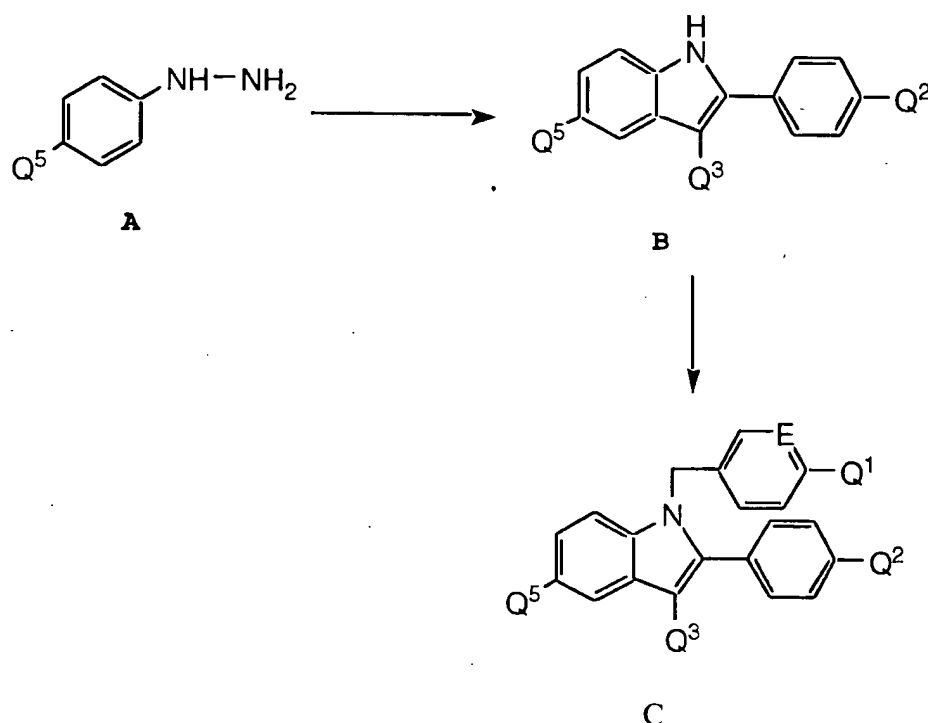
A particular value for a (1-3C)alkyl group is methyl, ethyl, propyl or isopropyl; for a (1-4C)alkoxy group is methoxy, ethoxy, isopropoxy or t-butoxy.

A compound of formula I may be made by processes
5 which include processes known in the chemical art for the production of known compounds of formula I or of structurally analogous compounds or by a novel process described herein. A process for a novel compound of formula I (or a pharmaceutically acceptable salt thereof),
10 novel processes for a compound of formula I and novel intermediates for the manufacture of a compound of formula I as defined above provide further features of the invention and are illustrated by the following procedures in which the meanings of the generic radicals are as defined above,
15 unless otherwise specified. It will be recognized that it may be preferred or necessary to prepare a compound of formula I in which a functional group is protected using a conventional protecting group, then to remove the protecting group to provide the compound of formula I.

20 In general, a compound of formula I may be prepared according to one of the routes outlined in Scheme I, and described in the examples, in which each of Q^1 , Q^2 , Q^3 and Q^5 , respectively, represents a value defined for the groups R^1 , R^2 , R^3 and R^5 , a protected version of
25 such a group, or moiety which can be further elaborated into such a group. Final conversion of a group Q^1 , Q^2 , Q^3 or Q^5 into R^1 , R^2 , R^3 or R^5 is carried out at a convenient point, consistent with the chemistry employed. It will be recognized that a number of other routes which are well
30 preceded in organic synthesis may be employed.

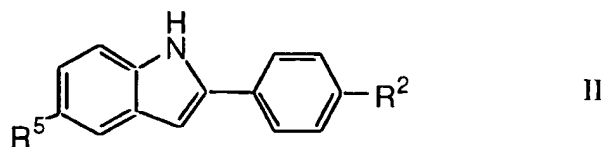
-9-

Scheme I

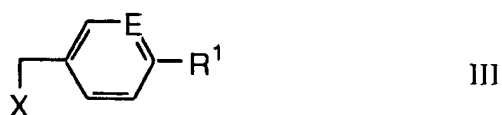


Thus, there is provided a process for preparing a novel compound of formula I (or a pharmaceutically acceptable salt thereof) as provided in any of the above descriptions which is selected from any of those described in the examples, including:

(a) For a compound of formula I in which R^3 is hydrogen, alkylating a compound of formula II



with an alkylating agent of formula III



-10-

in which X is a conventional leaving group, using a standard indole alkylation procedure, for example by using a procedure such as that described in Example 1-C, Example 7, Example 8-A or Example 11.

5 (b) For a compound of formula I in which R^1 or R^f is hydroxymethyl, reducing a corresponding compound of formula I in which R^1 or R^f is [(1-4C)alkoxy]carbonyl, for example by using a procedure such as that described in Example 1-D, Example 8-B or Example 12.

10 (c) For a compound of formula I in which R^1 or R^2 is $-X^1-(CH_2)_s-NR^sR^t$ or $-X^2-(CH_2)_m-NR^aR^b$, respectively, alkylating an amine of formula $H-NR^sR^t$ or $H-NR^aR^b$, respectively, with a compound corresponding to formula I, but in which R^1 or R^2 is $-X^1-(CH_2)_s-X$ or $-X^2-(CH_2)_m-X$,
15 respectively, in which X is a conventional leaving group, using a standard alkylation procedure, for example by using a procedure such as that described in Example 1-E, Example 6 or Example 8-C. The requisite alkylating agent is conveniently obtained from the corresponding alcohol by its conversion
20 into a leaving group, for example as described in the examples.

(d) For a compound of formula I in which R^3 is chloro, chlorinating a corresponding compound of formula I in which R^3 is hydrogen using a conventional procedure, for
25 example as described in the chlorination of the intermediate compound in Example 6.

(e) For a compound of formula I in which X^1 or X^2 is O, alkylating a phenolic compound corresponding to formula I, but in which R^1 or R^2 , respectively, is hydroxy using a
30 reagent of formula $X-(CH_2)_s-NR^sR^t$ or of formula $X-(CH_2)_m-NR^aR^b$ or $X-(CH_2)_n-R^f$, respectively, in which X is a conventional leaving group, using a standard alkylation procedure, for example by using a procedure such as that described in Example 8-D or Example 11.

35 (f) For a compound of formula I in which R^1 or R^f is carboxy, decomposing the ester of a corresponding compound of formula I in which R^1 or R^f is [(1-4C)alkoxy]carbonyl, for

-11-

example by using a procedure such as that described in Example 2 or Example 10.

(g) For a compound of formula I in which R³ is a benzyl group which may bear a methyl or methoxy substituent at the 3-position and a [(1-4C)alkoxy]carbonyl substituent at the 4-position, alkylating a corresponding compound of formula I in which R³ is hydrogen using a corresponding benzyl reagent bearing a leaving group X at the α -position using a conventional procedure, for example as described in the bis-alkylation of Example 1-C.

Whereafter, for any of the above procedures, when a functional group is protected using a protecting group, removing the protecting group.

Whereafter, for any of the above procedures, when a pharmaceutically acceptable salt of a compound of formula I is required, it may be obtained by reacting the basic form of such a compound of formula I with an acid affording a physiologically acceptable counterion or by reacting the acidic form of such a compound of formula I with a base affording a physiologically acceptable counterion or by any other conventional procedure.

As used herein, a leaving group "X" is a moiety which is displaced in a nucleophilic substitution reaction, for example a halo group (such as chloro, bromo or iodo), a sulfonate ester group (such as methylsulfonyloxy, p-toluylsulfonyloxy or trifluoromethylsulfonyloxy), or the reactive species derived from treating an alcohol with triphenylphosphine, diethyl azodicarboxylate and triethyl amine (in a Mitsunobu reaction).

Novel intermediate or starting material compounds, such as an indole of formula II provide a further aspect of the invention.

As mentioned above, a compound corresponding to a compound of formula I but in which a functional group is protected may serve as an intermediate for a compound of formula I. Accordingly, such protected intermediates for a novel compound of formula I provide further aspects of the

-12-

invention. Thus, as one particular aspect of the invention, there is provided a compound corresponding to a novel compound of formula I as defined above in which R⁵ which is hydroxy, but in which the corresponding substituent is -ORP in place of hydroxy, wherein RP is a phenol protecting group other than methyl. Phenol protecting groups are well known in the art, for example as described in T.W. Greene and P.G.M. Wuts, "Protecting Groups in Organic Synthesis" (1991). Particular values of RP include, for example, benzyl and allyl. Further, RP may denote a functionalized resin, for example as disclosed in H.V. Meyers, et al., Molecular Diversity, (1995), 1, 13-20.

As mentioned above, the invention includes pharmaceutically acceptable salts of the thrombin inhibiting compounds defined by the above formula I. A particular compound of this invention possesses one or more sufficiently basic functional groups to react with any of a number of inorganic and organic acids affording a physiologically acceptable counterion to form a pharmaceutically acceptable salt. Acids commonly employed to form pharmaceutically acceptable acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromobenzenesulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such pharmaceutically acceptable salts thus are the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate,

-13-

xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate, glycollate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate, and the like. Preferred pharmaceutically acceptable acid addition salts include those formed with mineral acids such as hydrochloric acid, hydrobromic acid and sulfuric acid.

If not commercially available, the necessary starting materials for the preparation of a compound of formula I may be prepared by procedures which are selected from standard techniques of organic chemistry, including aromatic and heteroaromatic substitution and transformation, from techniques which are analogous to the syntheses of known, structurally similar compounds, and techniques which are analogous to the above described procedures or procedures described in the Examples. It will be clear to one skilled in the art that a variety of sequences is available for the preparation of the starting materials. Starting materials which are novel provide another aspect of the invention.

Generally, the compounds of the invention are isolated best in the form of acid addition salts. Salts of the compounds of formula I formed with acids such as those mentioned above are useful as pharmaceutically acceptable salts for administration of the antithrombotic agents and for preparation of formulations of these agents. Other acid addition salts may be prepared and used in the isolation and purification of the compounds.

As noted above, the optically active isomers and diastereomers of the compounds of formula I are also considered part of this invention. Such optically active isomers may be prepared from their respective optically active precursors by the procedures described above, or by resolving the racemic mixtures. This resolution can be carried out by derivatization with a chiral reagent followed by chromatography or by repeated crystallization. Removal of the chiral auxiliary by standard methods affords

-14-

substantially optically pure isomers of the compounds of the present invention or their precursors. Further details regarding resolutions can be obtained in Jacques, et al., Enantiomers, Racemates, and Resolutions, John Wiley & Sons, 1981.

The compounds of the invention are believed to selectively inhibit thrombin over other proteinases and nonenzyme proteins involved in blood coagulation without appreciable interference with the body's natural clot lysing ability (the compounds have a low inhibitory effect on fibrinolysis). Further, such selectivity is believed to permit use with thrombolytic agents without substantial interference with thrombolysis and fibrinolysis.

The invention in one of its aspects provides a method of inhibiting thrombin in mammals comprising administering to a mammal in need of treatment an effective (thrombin inhibiting) dose of a compound of formula I.

In another of its aspects, the invention provides a method of treating a thromboembolic disorder comprising administering to a mammal in need of treatment an effective (thromboembolic disorder therapeutic and/or prophylactic amount) dose of a compound of formula I.

The invention in another of its aspects provides a method of inhibiting coagulation in mammals comprising administering to a mammal in need of treatment an effective (coagulation inhibiting) dose of a compound of formula I.

The thrombin inhibition, coagulation inhibition and thromboembolic disorder treatment contemplated by the present method includes both medical therapeutic and/or prophylactic treatment as appropriate.

In a further embodiment the invention relates to treatment, in a human or animal, of conditions where inhibition of thrombin is required. The compounds of the invention are expected to be useful in animals, including man, in treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues. Disorders in which the compounds have a potential utility are in treatment or

-15-

prophylaxis of thrombosis and hypercoagulability in blood and tissues. Disorders in which the compounds have a potential utility, in treatment and/or prophylaxis, include venous thrombosis and pulmonary embolism, arterial

5 thrombosis, such as in myocardial ischemia, myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis. Further, the compounds have expected utility in the treatment or prophylaxis of

10 atherosclerotic disorders (diseases) such as coronary arterial disease, cerebral arterial disease and peripheral arterial disease. Further, the compounds are expected to be useful together with thrombolytics in myocardial infarction. Further, the compounds have expected utility in prophylaxis for reocclusion after thrombolysis, percutaneous

15 transluminal angioplasty (PTCA) and coronary bypass operations. Further, the compounds have expected utility in prevention of rethrombosis after microsurgery. Further, the compounds are expected to be useful in anticoagulant treatment in connection with artificial organs and cardiac

20 valves. Further, the compounds have expected utility in anticoagulant treatment in hemodialysis and disseminated intravascular coagulation. A further expected utility is in rinsing of catheters and mechanical devices used in patients in vivo, and as an anticoagulant for preservation of blood,

25 plasma and other blood products in vitro. Still further, the compounds have expected utility in other diseases where blood coagulation could be a fundamental contributing process or a source of secondary pathology, such as cancer, including metastasis, inflammatory diseases, including

30 arthritis, and diabetes. The anti-coagulant compound is administered orally, parenterally e.g. by intravenous infusion (iv), intramuscular injection (im) or subcutaneously (sc).

The specific dose of a compound administered

35 according to this invention to obtain therapeutic and/or prophylactic effects will, of course, be determined by the particular circumstances surrounding the case, including,

-16-

for example, the compound administered, the rate of administration, the route of administration, and the condition being treated.

A typical daily dose for each of the above
5 utilities is between about 0.01 mg/kg and about 1000 mg/kg. The dose regimen may vary e.g. for prophylactic use a single daily dose may be administered or multiple doses such as 3 or 5 times daily may be appropriate. In critical care
10 situations a compound of the invention is administered by iv infusion at a rate between about 0.01 mg/kg/h and about 20 mg/kg/h and preferably between about 0.1 mg/kg/h and about 5 mg/kg/h.

The method of this invention also is practiced in conjunction with a clot lysing agent e.g. tissue plasminogen
15 activator (t-PA), modified t-PA, streptokinase or urokinase. In cases when clot formation has occurred and an artery or vein is blocked, either partially or totally, a clot lysing agent is usually employed. A compound of the invention can be administered prior to or along with the lysing agent or
20 subsequent to its use, and preferably further is administered along with aspirin to prevent the reoccurrence of clot formation.

The method of this invention is also practiced in conjunction with a platelet glycoprotein receptor (IIb/IIIa)
25 antagonist, that inhibits platelet aggregation. A compound of the invention can be administered prior to or along with the IIb/IIIa antagonist or subsequent to its use to prevent the occurrence or reoccurrence of clot formation.

The method of this invention is also practiced in
30 conjunction with aspirin. A compound of the invention can be administered prior to or along with aspirin or subsequent to its use to prevent the occurrence or reoccurrence of clot formation. As stated above, preferably a compound of the present invention is administered in conjunction with a clot
35 lysing agent and aspirin.

This invention also provides pharmaceutical formulations for use in the above described therapeutic

-17-

method. Pharmaceutical formulations of the invention comprise an effective thrombin inhibiting amount of a compound of formula I in association with a pharmaceutically acceptable carrier, excipient or diluent. For oral
5 administration the antithrombotic compound is formulated in gelatin capsules or tablets which may contain excipients such as binders, lubricants, disintegration agents and the like. For parenteral administration the antithrombotic is formulated in a pharmaceutically acceptable diluent e.g.
10 physiological saline (0.9 percent), 5 percent dextrose, Ringer's solution and the like.

The compound of the present invention can be formulated in unit dosage formulations comprising a dose between about 0.1 mg and about 1000 mg. Preferably the
15 compound is in the form of a pharmaceutically acceptable salt such as for example the sulfate salt, acetate salt or a phosphate salt. An example of a unit dosage formulation comprises 5 mg of a compound of the present invention as a pharmaceutically acceptable salt in a 10 mL sterile glass
20 ampoule. Another example of a unit dosage formulation comprises about 10 mg of a compound of the present invention as a pharmaceutically acceptable salt in 20 mL of isotonic saline contained in a sterile ampoule.

The compounds can be administered by a variety of
25 routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. The compounds of the present invention are preferably formulated prior to administration. Another embodiment of the present invention is a pharmaceutical formulation comprising an effective
30 amount of a novel compound of formula I or a pharmaceutically acceptable salt or solvate thereof in association with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The active ingredient in such formulations
35 comprises from 0.1 percent to 99.9 percent by weight of the formulation. By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with

-18-

the other ingredients of the formulation and not deleterious to the recipient thereof.

The present pharmaceutical formulations are prepared by known procedures using well known and readily available ingredients. The compositions of this invention may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art. In making the compositions of the present invention, the active ingredient will usually be admixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions, sterile packaged powders, and the like.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way. "Active ingredient," of course, means a compound according to formula I or a pharmaceutically acceptable salt or solvate thereof.

Formulation 1: Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active ingredient	250
Starch, dried	200
Magnesium stearate	<u>10</u>
Total	460 mg

-19-

Formulation 2: A tablet is prepared using the ingredients below:

	Quantity (mg/tablet)
Active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	<u>5</u>
Total	665 mg

5 The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3: An aerosol solution is prepared containing the following components:

	<u>Weight</u>
Active ingredient	0.25
Ethanol	25.75
Propellant 22 (Chlorodifluoromethane)	<u>70.00</u>
Total	100.00

10 The active compound is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to -30 °C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then
15 fitted to the container.

Formulation 4: Tablets, each containing 60 mg of active ingredient, are made as follows:

Active ingredient	60 mg
Starch	45 mg
Microcrystalline cellulose	35 mg
Polyvinylpyrrolidone (as 10 % solution in water)	4 mg
Sodium carboxymethyl starch	4.5 mg

-20-

Magnesium stearate	0.5 mg
Talc	<u>1 mg</u>
Total	150 mg

The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The aqueous solution containing polyvinylpyrrolidone is mixed with the resultant powder, and the mixture then is passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50 °C and passed through a No. 18 mesh U.S. Sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation 5: Capsules, each containing 80 mg of active ingredient, are made as follows:

Active ingredient	80 mg
Starch	59 mg
Microcrystalline cellulose	59 mg
Magnesium stearate	<u>2 mg</u>
Total	200 mg

The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6: Suppositories, each containing 225 mg of active ingredient, are made as follows:

Active ingredient	225 mg
Saturated fatty acid glycerides	<u>2,000 mg</u>
Total	2,225 mg

-21-

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The
5 mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

Formulation 7: Suspensions, each containing 50 mg of active ingredient per 5 ml dose, are made as follows:

10

Active ingredient	50 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mL
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to total	5 mL

15

The active ingredient is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution,
15 flavor and color are diluted with a portion of the water and added, with stirring. Sufficient water is then added to produce the required volume.

20

Formulation 8: An intravenous formulation may be prepared as follows:

Active ingredient	100 mg
Isotonic saline	1,000 mL

25

The solution of the above ingredients generally is administered intravenously to a subject at a rate of 1 mL
25 per minute.

The ability of the compounds of the present invention to be an effective and orally active thrombin

-22-

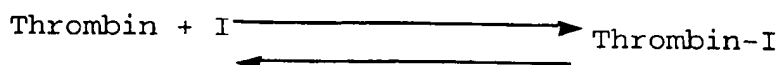
inhibitor are evaluated in one or more of the following assays.

The compounds provided by the invention (formula I) selectively inhibit the action of thrombin in mammals.

5 The inhibition of thrombin is demonstrated by *in vitro* inhibition of the amidase activity of thrombin as measured in an assay in which thrombin hydrolyzes the chromogenic substrate, N-benzoyl-L-phenylalanyl-L-valyl-L-arginyl-p-nitroanilide, N-benzoyl-L-Phe-L-Val-L-Arg-p-nitroanilide.

10 The assay is carried out by mixing 50 μ L buffer (0.03M Tris, 0.15M NaCl, pH 7.4) with 25 μ L of human thrombin solution (purified human thrombin, Enzyme Research Laboratories, South Bend, Indiana, at 8 NIH units/mL) and 25 μ L of test compound in a solvent (50% aqueous methanol (v:v)). Then 150 μ L of an aqueous solution of the
15 chromogenic substrate (at 0.25 mg/mL) are added and the rates of hydrolysis of the substrate are measured by monitoring the reactions at 405 nm for the release of p-nitroaniline. Standard curves are constructed by plotting free thrombin
20 concentration against hydrolysis rate. The hydrolysis rates observed with test compounds are then converted to "free thrombin" values in the respective assays by use of the standard curves. The bound thrombin (bound to test compound) is calculated by subtracting the amount of free
25 thrombin observed in each assay from the known initial amount of thrombin used in the assay. The amount of free inhibitor in each assay is calculated by subtracting the number of moles of bound thrombin from the number of moles of added inhibitor (test compound).

30 The Kass value is the hypothetical equilibrium constant for the reaction between thrombin and the test compound (I).



$$\text{Kass} = \frac{[\text{Thrombin-I}]}{[(\text{Thrombin}) \times (\text{I})]}$$

-23-

Kass is calculated for a range of concentrations of test compounds and the mean value reported in units of liter per mole. In general, a thrombin inhibiting compound of formula I of the instant invention exhibits a Kass of 0.03×10^6 L/mole or much greater.

By substantially following the procedures described above for human thrombin, and using other human blood coagulation system serine proteases and using fibrinolytic system serine proteases, with the appropriate chromogenic substrates, identified below, the selectivity of the compounds of the present invention with respect to the coagulation factor serine proteases and to the fibronolytic serine proteases are evaluated as well as their substantial lack of interference with human plasma clot fibrinolysis.

Human factors X, Xa, IXa, XIa, and XIIa are purchased from Enzyme Research Laboratories, South Bend, Indiana; human urokinase from Leo Pharmaceuticals, Denmark; and recombinant activated Protein C (aPC) is prepared at Eli Lilly and Co. substantially according to U.S. Patent 4,981,952. Chromogenic substrates: N-Benzoyl-Ile-Glu-Gly-Arg-p-nitroanilide (for factor Xa); N-Cbz-D-Arg-Gly-Arg-p-nitroanilide (for factor IXa assay as the factor Xa substrate); Pyroglutamyl-Pro-Arg-p-nitroanilide (for Factor XIa and for aPC); H-D-Pro-Phe-Arg-p-nitroanilide (for factor XIIa); and Pyroglutamyl-Gly-Arg-p-nitroanilide (for urokinase); are purchased from Kabi Vitrum, Stockholm, Sweden, or from Midwest Biotech, Fishers, Indiana. Bovine trypsin is purchased from Worthington Biochemicals, Freehold, New Jersey, and human plasma kallikrein from Kabi Vitrum, Stockholm, Sweden. Chromogenic substrate H-D-Pro-Phe-Arg-p-nitroanilide for plasma kallikrein is purchased from Kabi Vitrum, Stockholm, Sweden. N-Benzoyl-Phe-Val-Arg-p-nitroanilide, the substrate for human thrombin and for trypsin, is synthesized according to procedures described above for the compounds of the present invention, using known methods of peptide coupling from commercially

-24-

available reactants, or purchased from Midwest Biotech, Fishers, Indiana.

Human plasmin is purchased from Boehringer Mannheim, Indianapolis, Indiana; nt-PA is purchased as
5 single chain activity reference from American Diagnostica, Greenwich, Connecticut; modified-t-PA6 (mt-PA6) is prepared at Eli Lilly and Company by procedure known in the art (See, Burck, et al., J. Biol. Chem., 265, 5120-5177 (1990)).

10 Plasmin chromogenic substrate H-D-Val-Leu-Lys-p-nitroanilide and tissue plasminogen activator (t-PA) substrate H-D-Ile-Pro-Arg-p-nitroanilide are purchased from Kabi Vitrum, Stockholm, Sweden.

In the chromogenic substrates described above the three-letter symbols Ile, Glu, Gly, Pro, Arg, Phe, Val, Leu
15 and Lys are used to indicate the corresponding amino acid group isoleucine, glutamic acid, glycine, proline, arginine, phenylalanine, valine, leucine and lysine, respectively.

Thrombin inhibitors preferably should spare fibrinolysis induced by urokinase, tissue plasminogen
20 activator (t-PA) and streptokinase. This would be important to the therapeutic use of such agents as an adjunct to streptokinase, t-PA or urokinase thrombolytic therapy and to the use of such agents as an endogenous fibrinolysis-sparing (with respect to t-PA and urokinase) antithrombotic agents.
25 In addition to the lack of interference with the amidase activity of the fibrinolytic proteases, such fibrinolytic system sparing can be studied by the use of human plasma clots and their lysis by the respective fibrinolytic plasminogen activators.

30

Materials

Dog plasma is obtained from conscious mixed-breed hounds (either sex Butler Farms, Clyde, New York, U.S.A.) by
venipuncture into 3.8 percent citrate. Fibrinogen is
35 prepared from fresh dog plasma and human fibrinogen is prepared from in-date ACD human blood at the fraction I-2 according to previous procedures and specifications. Smith,

-25-

Biochem. J., 185, 1-11 (1980); and Smith, et al.,
Biochemistry, 11, 2958-2967, (1972). Human fibrinogen (98
percent pure/plasmin free) is from American Diagnostica,
Greenwich, Connecticut. Radiolabeling of fibrinogen I-2
5 preparations is performed as previously reported. Smith, et
al., Biochemistry, 11, 2958-2967, (1972). Urokinase is
purchased from Leo Pharmaceuticals, Denmark, as 2200 Ploug
units/vial. Streptokinase is purchased from Hoechst-Roussel
Pharmaceuticals, Somerville, New Jersey.

10

Methods - Effects on Lysis of Human Plasma Clots by t-PA

Human plasma clots are formed in micro test tubes by adding
50 μ L thrombin (73 NIH unit/mL) to 100 μ L human plasma which
contains 0.0229 μ Ci 125-iodine labeled fibrinogen. Clot
15 lysis is studied by overlaying the clots with 50 μ L of
urokinase or streptokinase (50, 100, or 1000 unit/mL) and
incubating for 20 hours at room temperature. After
incubation the tubes are centrifuged in a Beckman Microfuge.
25 μ L of supernate is added into 1.0 mL volume of 0.03 M
20 tris/0.15 M NaCl buffer for gamma counting. Counting
controls 100 percent lysis are obtained by omitting thrombin
(and substituting buffer). The thrombin inhibitors are
evaluated for possible interference with fibrinolysis by
including the compounds in the overlay solutions at 1, 5,
25 and 10 μ g/mL concentrations. Rough approximations of IC₅₀
values are estimated by linear extrapolations from data
points to a value which would represent 50 percent of lysis
for that particular concentration of fibrinolytic agent.

30 Anticoagulant Activity

Materials

Dog plasma and rat plasma are obtained from conscious mixed-
breed hounds (either sex, Butler Farms, Clyde, New York,
U.S.A.) or from anesthetized male Sprague-Dawley rats
35 (Harlan Sprague-Dawley, Inc., Indianapolis, Indiana, U.S.A.)
by venipuncture into 3.8 percent citrate. Fibrinogen is
prepared from in-date ACD human blood as the fraction I-2

-26-

according to previous procedures and specifications. Smith, Biochem. J., 185, 1-11 (1980); and Smith, et al., Biochemistry, 11, 2958-2967 (1972). Human fibrinogen is also purchased as 98 percent pure/plasmin free from American Diagnostica, Greenwich, Connecticut. Coagulation reagents Actin, Thromboplastin, Innovin and Human plasma are from Baxter Healthcare Corp., Dade Division, Miami, Florida. Bovine thrombin from Parke-Davis (Detroit, Michigan) is used for coagulation assays in plasma.

10

Methods

Anticoagulation Determinations

Coagulation assay procedures are as previously described. Smith, et al., Thrombosis Research, 50, 163-174 (1988). A CoAScreener coagulation instrument (American LABor, Inc.) is used for all coagulation assay measurements. The prothrombin time (PT) is measured by adding 0.05 mL saline and 0.05 mL Thromboplastin-C reagent or recombinant human tissue factor reagent (Innovin) to 0.05 mL test plasma. The activated partial thromboplastin time (APTT) is measured by incubation of 0.05 mL test plasma with 0.05 mL Actin reagent for 120 seconds followed by 0.05 mL CaCl₂ (0.02 M). The thrombin time (TT) is measured by adding 0.05 mL saline and 0.05 mL thrombin (10 NIH units/mL) to 0.05 mL test plasma. The compounds of formula I are added to human or animal plasma over a wide range of concentrations to determine prolongation effects on the APTT, PT, and TT assays. Linear extrapolations are performed to estimate the concentrations required to double the clotting time for each assay.

30

Animals

Male Sprague Dawley rats (350-425 gm, Harlan Sprague Dawley Inc., Indianapolis, IN) are anesthetized with xylazine (20 mg/kg, s.c.) and ketamine (120 mg/kg, s.c.) and maintained on a heated water blanket (37 °C). The jugular vein(s) is cannulated to allow for infusions.

35

-27-

Arterio-Venous shunt model

The left jugular vein and right carotid artery are cannulated with 20 cm lengths of polyethylene PE 60 tubing. A 6 cm center section of larger tubing (PE 190) with a cotton thread (5 cm) in the lumen, is friction fitted between the longer sections to complete the arterio-venous shunt circuit. Blood is circulated through the shunt for 15 min before the thread is carefully removed and weighed. The weight of a wet thread is subtracted from the total weight of the thread and thrombus (see J.R. Smith, Br J Pharmacol, 77:29, 1982). In this model preferred compounds of the instant invention reduce the net clot weight to approximately 25-30% of control, or even lower, at an i.v. dose of 33.176 $\mu\text{mol/kg/h}$.

FeCl₃ model of arterial injury

The carotid arteries are isolated via a midline ventral cervical incision. A thermocouple is placed under each artery and vessel temperature is recorded continuously on a strip chart recorder. A cuff of tubing (0.058 ID x 0.077 OD x 4 mm, Baxter Med. Grade Silicone), cut longitudinally, is placed around each carotid directly above the thermocouple. FeCl₃ hexahydrate is dissolved in water and the concentration (20 percent) is expressed in terms of the actual weight of FeCl₃ only. To injure the artery and induce thrombosis, 2.85 μL is pipetted into the cuff to bathe the artery above the thermocouple probe. Arterial occlusion is indicated by a rapid drop in temperature. The time to occlusion is reported in minutes and represents the elapsed time between application of FeCl₃ and the rapid drop in vessel temperature (see K.D. Kurz, Thromb. Res., 60:269, 1990).

Spontaneous thrombolysis model

In vitro data suggests that thrombin inhibitors inhibit thrombin and, at higher concentrations, may inhibit other serine proteases, such as plasmin and tissue plasminogen

-28-

activator. To assess if the compounds inhibit fibrinolysis in vivo, the rate of spontaneous thrombolysis is determined by implanting a labeled whole blood clot into the pulmonary circulation. Rat blood (1 mL) is mixed rapidly with bovine thrombin (4 IU, Parke Davis) and ^{125}I human Fibrogen (5 μCi , ICN), immediately drawn into silastic tubing and incubated at 37 °C for 1 hour. The aged thrombus is expelled from the tubing, cut into 1 cm segments, washed 3X in normal saline and each segment is counted in a gamma counter. A segment with known counts is aspirated into a catheter that is subsequently implanted into the jugular vein. The catheter tip is advanced to the vicinity of the right atrium and the clot is expelled to float into the pulmonary circulation. One hour after implant, the heart and lungs are harvested and counted separately. Thrombolysis is expressed as a percentage where:

$$\% \text{ Thrombolysis} = \frac{(\text{injected cpm} - \text{lung cpm})}{\text{injected cpm}} \times 100$$

The fibrinolytic dissolution of the implanted clot occurs time-dependently (see J.P. Clozel, Cardiovas. Pharmacol., 12:520, 1988).

25 Coagulation parameters

Plasma thrombin time (TT) and activated partial thromboplastin time (APTT) are measured with a fibrometer. Blood is sampled from a jugular catheter and collected in syringe containing sodium citrate (3.8 percent, 1 part to 9 parts blood). To measure TT, rat plasma (0.1 mL) is mixed with saline (0.1 mL) and bovine thrombin (0.1 mL, 30 U/mL in TRIS buffer; Parke Davis) at 37 °C. For APTT, plasma (0.1 mL) and APTT solution (0.1 mL, Organon Teknika) are incubated for 5 minutes (37 °C) and CaCl_2 (0.1 mL, 0.025 M) is added to start coagulation. Assays are done in duplicate and averaged.

-29-

Index of Bioavailability

For a measure of bioactivity, plasma thrombin time (TT) serves as a substitute for the assay of parent compound on the assumption that observed increments in TT resulted from thrombin inhibition by parent only. The time course of the effect of the thrombin inhibitor upon TT is determined after i.v bolus administration to anesthetized rats and after oral treatment of fasted conscious rats. Due to limitations of blood volume and the number of points required to determine the time course from time of treatment to the time when the response returns to pretreatment values, two populations of rats are used. Each sample population represents alternating sequential time points. The average TT over the time course is used to calculate area under the curve (AUC). The index of bioavailability is calculated by the formula shown below and is expressed as percent relative activity.

The area under the curve (AUC) of the plasma TT time course is determined and adjusted for the dose. This index of bioavailability is termed "% Relative Activity" and is calculated as

$$\% \text{ Relative Activity} = \frac{\text{AUC po}}{\text{AUC iv}} \times \frac{\text{Dose iv}}{\text{Dose po}} \times 100$$

Compounds

Compound solutions are prepared fresh daily in normal saline and are injected as a bolus or are infused starting 15 minutes before and continuing throughout the experimental perturbation which is 15 minutes in the arteriovenous shunt model and 60 minutes in the FeCl₃ model of arterial injury and in the spontaneous thrombolysis model. Bolus injection volume is 1 mL/kg for i.v., and 5 mL/kg for p.o., and infusion volume is 3 mL/hr.

-30-

Statistics

Results are expressed as means \pm SEM. One-way analysis of variance is used to detect statistically significant differences and then Dunnett's test is applied to determine which means are different. Significance level for rejection of the null hypothesis of equal means is $P < 0.05$.

Animals

Male dogs (Beagles; 18 months - 2 years; 12-13 kg, Marshall Farms, North Rose, New York 14516) are fasted overnight and fed Purina certified Prescription Diet (Purina Mills, St. Louis, Missouri) 240 minutes after dosing. Water is available *ad libitum*. The room temperature is maintained between 66-74 °F; 45-50 percent relative humidity; and lighted from 0600-1800 hours.

Pharmacokinetic model.

Test compound is formulated immediately prior to dosing by dissolving in sterile 0.9 percent saline to a 5 mg/mL preparation. Dogs are given a single 2 mg/kg dose of test compound by oral gavage. Blood samples (4.5 mL) are taken from the cephalic vein at 0.25, 0.5, 0.75, 1, 2, 3, 4 and 6 hours after dosing. Samples are collected in citrated Vacutainer tubes and kept on ice prior to reduction to plasma by centrifugation. Plasma samples are analyzed by HPLC MS. Plasma concentration of test compound is recorded and used to calculate the pharmacokinetic parameters: elimination rate constant, K_e ; total clearance, Cl_t ; volume of distribution, V_D ; time of maximum plasma test compound concentration, T_{max} ; maximum concentration of test compound of T_{max} , C_{max} ; plasma half-life, $t_{0.5}$; and area under the curve, A.U.C.; fraction of test compound absorbed, F .

Canine Model of Coronary Artery Thrombosis

Surgical preparation and instrumentation of the dogs are as described in Jackson, et al., Circulation, 82, 930-940 (1990). Mixed-breed hounds (aged 6-7 months, either sex,

-31-

Butler Farms, Clyde, New York, U.S.A.) are anesthetized with sodium pentobarbital (30 mg/kg intravenously, i.v.), intubated, and ventilated with room air. Tidal volume and respiratory rates are adjusted to maintain blood PO_2 , PCO_2 , and pH within normal limits. Subdermal needle electrodes are inserted for the recording of a lead II ECG.

The left jugular vein and common carotid artery are isolated through a left mediolateral neck incision. Arterial blood pressure (ABP) is measured continuously with a precalibrated Millar transducer (model MPC-500, Millar Instruments, Houston, TX, U.S.A.) inserted into the carotid artery. The jugular vein is cannulated for blood sampling during the experiment. In addition, the femoral veins of both hindlegs are cannulated for administration of test compound.

A left thoracotomy is performed at the fifth intercostal space, and the heart is suspended in a pericardial cradle. A 1- to 2-cm segment of the left circumflex coronary artery (LCX) is isolated proximal to the first major diagonal ventricular branch. A 26-gauge needle-tipped wire anodal electrode (Teflon-coated, 30-gauge silverplated copper wire) 3-4 mm long is inserted into the LCX and placed in contact with the intimal surface of the artery (confirmed at the end of the experiment). The stimulating circuit is completed by placing the cathode in a subcutaneous (s.c.) site. An adjustable plastic occluder is placed around the LCX, over the region of the electrode. A precalibrated electromagnetic flow probe (Carolina Medical Electronics, King, NC, U.S.A.) is placed around the LCX proximal to the anode for measurement of coronary blood flow (CBF). The occluder is adjusted to produce a 40-50 percent inhibition of the hyperemic blood flow response observed after 10-s mechanical occlusion of the LCX. All hemodynamic and ECG measurements are recorded and analyzed with a data acquisition system (model M3000, Modular Instruments, Malvern, PA, U.S.A.).

-32-

Thrombus Formation and Compound Administration Regimens

Electrolytic injury of the intima of the LCX is produced by applying 100- μ A direct current (DC) to the anode. The
5 current is maintained for 60 min and then discontinued whether the vessel has occluded or not. Thrombus formation proceeds spontaneously until the LCX is totally occluded (determined as zero CBF and an increase in the S-T segment). Compound administration is started after the occluding
10 thrombus is allowed to age for 1 hour. A 2-hour infusion of the compounds of the present invention at doses of 0.5 and 1 mg/kg/hour is begun simultaneously with an infusion of thrombolytic agent (e.g. tissue plasminogen activator, streptokinase, APSAC). Reperfusion is followed for 3 hour
15 after administration of test compound. Reocclusion of coronary arteries after successful thrombolysis is defined as zero CBF which persisted for at least 30 minutes.

Hematology and template bleeding time determinations

20 Whole blood cell counts, hemoglobin, and hematocrit values are determined on a 40- μ L sample of citrated (3.8 percent) blood (1 part citrate:9 parts blood) with a hematology analyzer (Cell-Dyn 900, Sequoia-Turner. Mount View, CA, U.S.A.). Gingival template bleeding times are determined
25 with a Simplate II bleeding time device (Organon Teknika Durham, N.C., U.S.A.). The device is used to make 2 horizontal incisions in the gingiva of either the upper or lower left jaw of the dog. Each incision is 3 mm wide x 2 mm deep. The incisions are made, and a stopwatch is used to
30 determine how long bleeding occurs. A cotton swab is used to soak up the blood as it oozes from the incision. Template bleeding time is the time from incision to stoppage of bleeding. Bleeding times are taken just before
35 administration of test compound (0 min), 60 min into infusion, at conclusion of administration of the test compound (120 min), and at the end of the experiment.

-33-

All data are analyzed by one-way analysis of variance (ANOVA) followed by Student-Neuman-Kuels post hoc *t* test to determine the level of significance. Repeated-measures ANOVA are used to determine significant differences between
5 time points during the experiments. Values are determined to be statistically different at least at the level of $p < 0.05$. All values are mean \pm SEM. All studies are conducted in accordance with the guiding principles of the American Physiological Society. Further details regarding
10 the procedures are described in Jackson, et al., J. Cardiovasc. Pharmacol., (1993), 21, 587-599.

The following Examples are provided to further describe the invention and are not to be construed as limitations thereof.

15 The abbreviations, symbols and terms used in the examples have the following meanings.

Ac = acetyl
AIBN = azobisisobutyronitrile
Anal. = elemental analysis
20 Bn or Bzl = benzyl
Bu = butyl
n-BuLi = butyllithium
calcd = calculated
DCC = dicyclohexylcarbodiimide
25 DIBAL-H = diisobutyl aluminum hydride
DMF = dimethylformamide
DMSO = dimethylsulfoxide
Et = ethyl
EtOAc = ethyl acetate
30 Et₃N = triethylamine
Et₂O = diethyl ether
EtOH = ethanol
EtSH = ethanethiol
FAB = Fast Atom Bombardment (Mass Spectrascopy)
35 FDMS = field desorption mass spectrum
Hex = hexanes
HOAt = 1-hydroxy-7-azabenzotriazole

-34-

HPLC = High Performance Liquid Chromatography

HRMS = high resolution mass spectrum

i-PrOH = isopropanol

IR = Infrared Spectrum

5 LAH = lithium aluminum hydride

Me = methyl

MeI = methyl iodide

MeOH = methanol

MPLC = Medium Pressure Liquid Chromatography

10 NBS = N-bromosuccinimide

NMR = Nuclear Magnetic Resonance

Ph = phenyl

PPA = polyphosphoric acid

i-Pr = isopropyl

15 Rochelle's Salt = potassium sodium tartrate

RPHPLC = Reversed Phase High Performance Liquid
Chromatography

SiO₂ = silica gel

SM = starting material

20 TBS = tert-butyldimethylsilyl

TEA = triethylamine

Temp. = temperature

TFA = trifluoroacetic acid

THF = tetrahydrofuran

25 TIPS = triisopropylsilyl

TLC = thin layer chromatography

triflic acid = trifluoromethanesulfonic acid

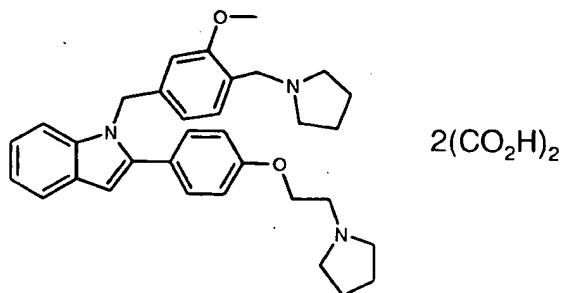
30 Unless otherwise stated, pH adjustments and work
up are with aqueous acid or base solutions. PrepLC
indicates preparative liquid chromatography using "Prep Pak
(TM)" silica cartridges; radial chromatography indicates
preparative chromatography using a "Chromatotron (TM)"
instrument.

-35-

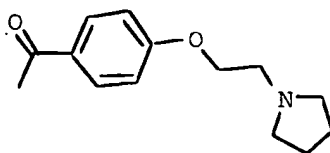
Example 1

Preparation of 1-[3-Methoxy-4-(1-pyrrolidinyl)methyl]benzyl-
2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indole Dioxalate.

5



A. Methyl 4-[2-(1-Pyrrolidinyl)ethoxy]phenyl Ketone.



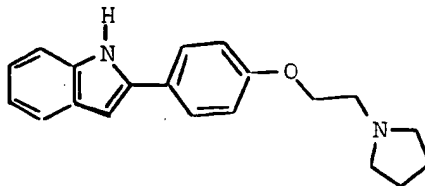
10

The title compound was prepared from 4'-hydroxy-acetophenone (6.80 g, 50 mmol) and 2-(1-pyrrolidinyl)ethanol (5.75 g, 50 mmol) by a Mitsunobu method in 69% yield.

15 FDMS 233 (M⁺); Anal. Calcd for C₁₄H₁₉NO₂·0.06 CH₂Cl₂:
C, 70.84; H, 8.08; N, 5.88. Found: C, 70.75; H, 7.88;
N, 5.66.

B. 2-[4-[2-(1-Pyrrolidinyl)ethoxy]phenyl]indole.

20



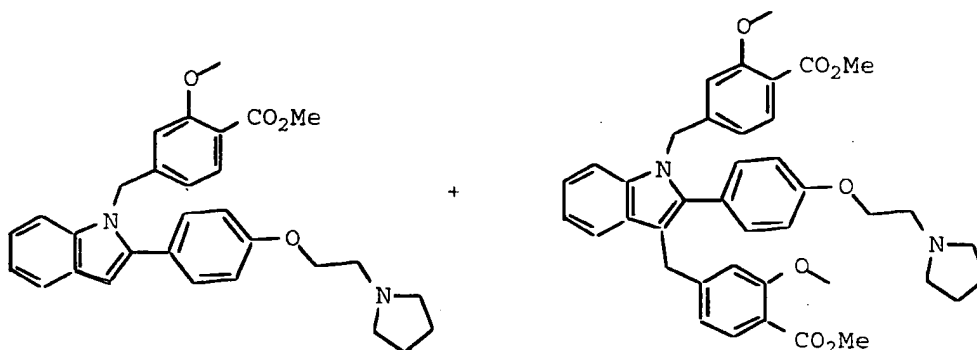
A mixture of phenylhydrazine (1.0 mL, 10 mmol),
acetophenone (Part A; 2.35 g, 10 mmol), and polyphosphoric
25 acid (ca. 5 g) in chlorobenzene (50 mL) was heated at ~145

-36-

°C (bath temp) for 1 h. The chlorobenzene layer was decanted off and the tar-like residue was stirred in water (100 mL) overnight. The resultant white suspension was treated with 2.0 N NaOH to pH 8-9. The mixture was taken up in EtOAc (250 mL) and washed with saturated aqueous NaHCO₃ and brine (100 mL each). The aqueous layers were back-extracted with EtOAc (250 mL x 2). The chlorobenzene layer was also washed with the same aqueous solution. Combined organic layers were dried over MgSO₄, concentrated, and purified by PrepLC with 35:6:59 THF-Et₃N-hexanes to afford 2.27 g (74%) of the title compound.

mp 173-177 °C; FDMS 306.3 (M+1); Anal. Calcd for C₂₀H₂₂N₂O: C, 78.40; H, 7.24; N, 9.14. Found: C, 78.26; H, 7.06; N, 8.97.

C. Methyl 2-Methoxy-4-[[2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indol-1-yl]methyl]benzoate and Methyl 2-Methoxy-4-[[3-[4-(3-methoxy-4-methoxycarbonyl)benzyl]-2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indol-1-yl]methyl]benzoate.



A mixture of the above indole (1.599 g, 5.2 mmol) and 60% NaH in oil dispersion (251 mg; 6.3 mmol) in anhydrous DMF (50 mL) was stirred at room temperature for 1 h. To the anionic solution at 0 °C was then cannulated methyl 4-bromo-2-methoxybenzoate (1.3566 g, 5.2 mmol) in 10 mL of DMF. After stirring at room temperature for 2 h, the reaction was quenched with 40 mL of H₂O at 0 °C. The

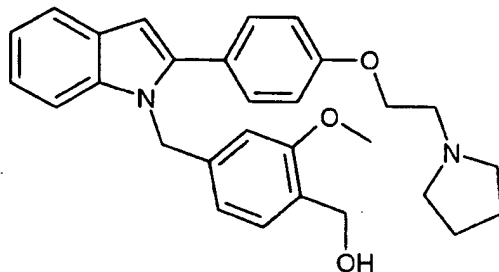
-37-

mixture was taken up in 200 mL of EtOAc and washed with H₂O (100 mL x 2) and brine (100 mL). The aqueous layers were back-extracted with EtOAc (200 mL x 2). Combined extracts were dried over MgSO₄, concentrated and purified by flash chromatography to afford 955.8 mg (38%) the title monoester, 586.3 mg of the diester, and 574.5 mg (36%) of the starting indole.

monoester: mp 127-130 °C; FDMS 483.8 (M⁺); Anal. Calcd for C₃₀H₃₂N₂O₄: C, 74.36; H, 6.66; N, 5.78. Found: C, 74.24; H, 6.42; N, 5.79.

diester: mp 45-49 °C; FDMS 662 (M⁺); Anal. Calcd for C₄₀H₄₂N₂O₇: C, 72.49; H, 6.39; N, 4.23. Found: C, 72.21; H, 6.29; N, 4.51.

D. 2-Methoxy-4-[[2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]-indol-1-yl]methyl]benzyl Alcohol.



A solution of the above monoester (506.6 mg, 1.05 mmol) in 5.0 mL of anhydrous THF was treated with 1.05 mL of 1.0 M LAH in THF at 0 °C for 2 h. After the standard Fieser work-up, a quantitative yield of the title compound (484 mg) was obtained.

mp 36-43 °C; FDMS 456 (M⁺); Anal. Calcd for C₂₉H₃₂N₂O₃·0.25C₄H₈O₂: C, 75.29; H, 7.16; N, 5.85. Found: C, 75.34; H, 7.28; N, 5.90.

E. 1-[3-Methoxy-4-(1-pyrrolidinyl)methyl]benzyl-2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indole Dioxalate.

-38-

An ice-cold solution of the above alcohol (119.3 mg, 0.26 mmol), Et₃N (73 μ L, 0.52 mmol), and mesyl chloride (20 μ L, 0.26 mmol) in 2.0 mL of THF was stirred for 2 h 20 min.

5 To this was then added pyrrolidine (0.22 mL, 2.6 mmol), and the mixture was stirred at room temperature for 19 h. The mixture was taken up in 25 mL of CH₂Cl₂ and washed with saturated aqueous NaHCO₃ and brine (20 mL each). The aqueous layers were back-extracted with CH₂Cl₂ (25 mL x 2).

10 Combined organic layers were dried over MgSO₄, concentrated, and flash chromatographed with 8:92 (10% conc NH₄OH in MeOH)-CH₂Cl₂ to afford 81.9 mg (62%) of the free base of the title compound. The title compound was prepared in a quantitative yield from the free base by formation of the

15 oxalate salt by dissolution in EtOH, and filtration and drying of the resultant solid.

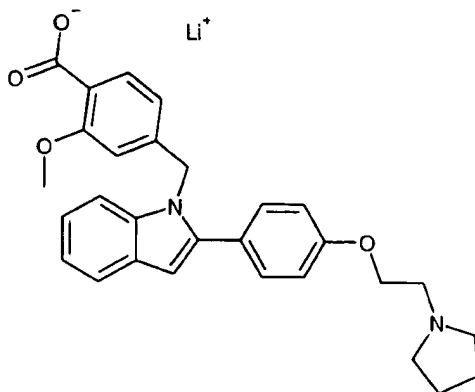
FDMS 510 (M⁺); Anal. Calcd for C₃₇H₄₃N₃O₁₀·0.65C₄H₈O₂: C, 63.67; H, 6.50; N, 5.62. Found: C, 63.72; H, 6.89; N, 5.73.

20

Example 2

Preparation of Lithium 2-Methoxy-4-[[2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indol-1-yl]methyl]benzoate.

25



Hydrolysis of the monoester of Example 1-C (122.0 mg, 0.25 mmol) with 0.5 N LiOH afforded 92% of the desired lithium carboxylate.

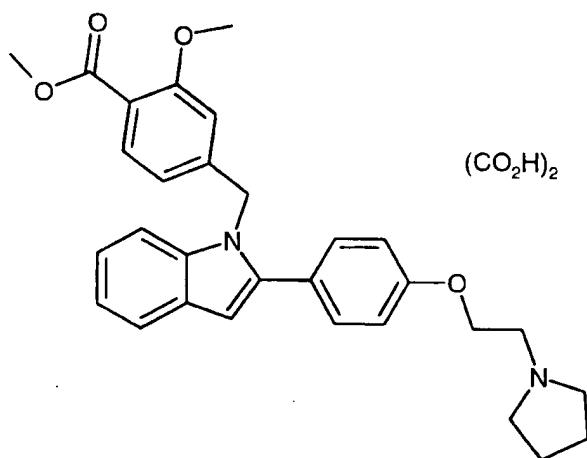
-39-

FDMS 477.3 (M+1); Anal. Calcd for $C_{29}H_{29}N_2O_4Li \cdot 1.3H_2O$: C, 69.68; H, 6.37; N, 5.60. Found: C, 69.67; H, 6.19; N, 5.67.

5

Example 3

Preparation of Methyl 2-Methoxy-4-[[2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indol-1-yl]methyl]benzoate Oxalate.



10

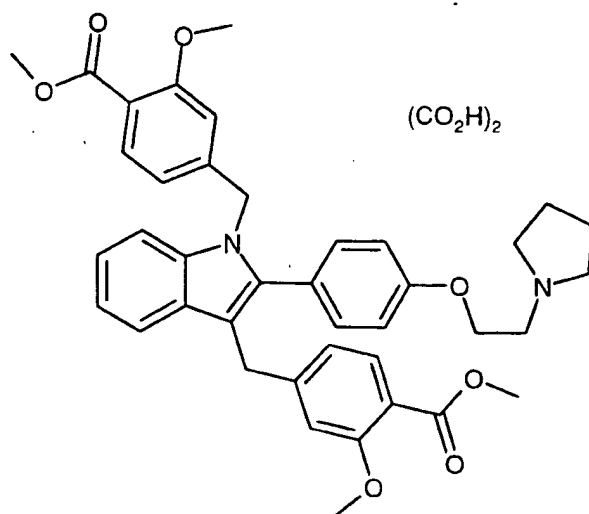
The title oxalate salt was prepared from the monoester of Example 1-C (97 mg, 0.20 mmol) by the method of Example 1-E in 27% yield.

15 FDMS 484 (M+); Anal. Calcd for $C_{30}H_{32}N_2O_4 \cdot 0.85C_2H_2O_4$: C, 67.85; H, 6.05; N, 4.99. Found: C, 67.85; H, 5.96; N, 4.86.

Example 4

20 Preparation of Methyl 2-Methoxy-4-[[3-[4-(3-methoxy-4-methoxycarbonyl)benzyl]-2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indol-1-yl]methyl]benzoate Oxalate.

-40-



The title oxalate salt was prepared from the diester of Example 1-C (127.7 mg, 0.19 mmol) by the method of Example 1-E in 29% yield.

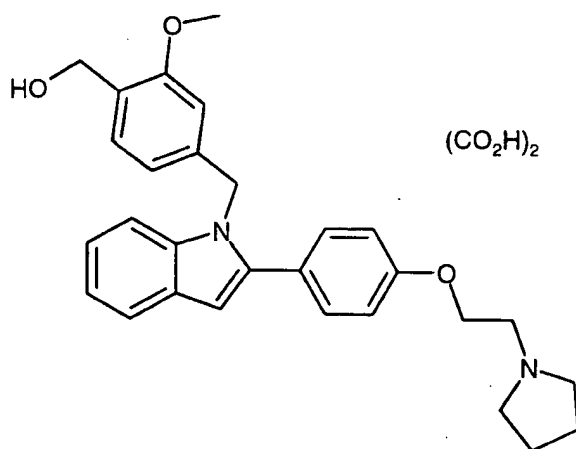
5

FDMS 662.1 (M⁺); Anal. Calcd for C₄₀H₄₂N₂O₇·0.83C₂H₂O₄: C, 67.85; H, 5.97; N, 3.80. Found: C, 67.83; H, 5.99; N, 3.98.

10

Example 5

Preparation of 2-Methoxy-4-[[2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indol-1-yl]methyl]benzyl Alcohol Oxalate.



15

The title oxalate salt was prepared from the alcohol of Example 1-D (114.2 mg, 0.25 mmol) by the method of Example 1-E in 72% yield.

-41-

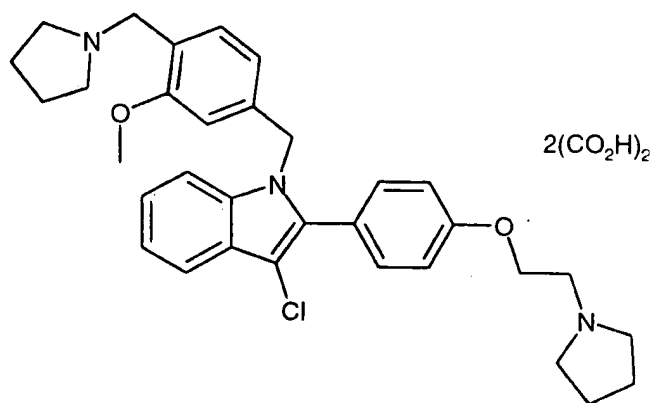
FDMS 456.1 (M⁺); Anal. Calcd for C₃₁H₃₄N₂O₇·1.13C₂H₂O₄: C, 67.25; H, 6.18; N, 5.02. Found: C, 67.26; H, 6.44; N, 4.88.

5

Example 6

Preparation of 3-Chloro-1-[3-methoxy-4-(1-pyrrolidinyl)-methyl]benzyl-2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indole Dioxalate.

10



A solution of the alcohol of Example 1-D (235.8 mg, 0.52 mmol) in 1.0 mL of CH₂Cl₂ was treated with SOCl₂ (75 μL, 1.0 mmol) at room temperature for 3.5 h, followed by another 37.5 mL of SOCl₂ addition and stirring for 1 h. The solvent and excess SOCl₂ was removed azeotropically with benzene under reduced pressure and the residue was dried under vacuum for 30 min. The dichloride formed was dissolved in 1.0 mL of anhydrous DMF and treated with 0.22 mL (2.6 mmol) of pyrrolidine at room temperature for 1 h. The reaction was quenched with 1.5 mL of saturated aqueous NaHCO₃. The mixture was taken up in 30 mL of EtOAc and washed with H₂O (20 mL x 2) and brine (20 mL). The aqueous layers were back-extracted with EtOAc (30 mL x 2). Combined organic layers dried over MgSO₄, concentrated and purified by flash chromatography with 8:92 (10% conc NH₄OH in MeOH)-CH₂Cl₂ to yield 91.4 mg (35%) of the free base of the title compound. The oxalate salt was formed in 59% yield by the method of Example 1-E.

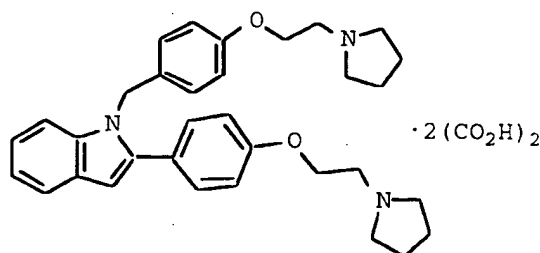
-42-

FDMS 544 (M+); Anal. Calcd for

C₃₇H₄₂N₃O₁₀Cl·0.4C₂H₂O₄·0.2C₄H₈O₂: C, 59.60; H, 5.75; N, 5.40; Cl, 4.56. Found: C, 59.25; H, 6.14; N, 5.56; Cl, 4.85.

Example 7

Preparation of 1-[4-[2-(1-pyrrolidinyl)ethoxy]benzyl]-2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indole Dioxalate.



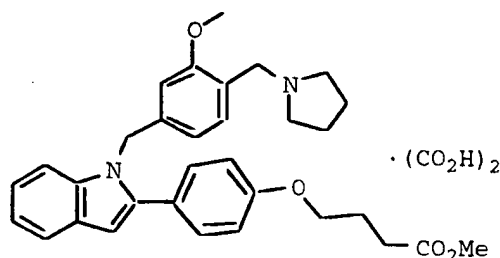
A mixture of 2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]-indole (309.8 mg, 1.0 mmol) and 170.2 mg (3.0 mmol) of KOH in 2.0 mL of anhydrous DMSO was stirred at room temperature for 5-8 min. To this was added in one portion 4-[2-(1-pyrrolidinyl)ethoxy]benzyl chloride hydrochloride (280 mg, 1.0 mmol) and the mixture was stirred for 70 min. The reaction was quenched with 20 mL of H₂O and the mixture was extracted with EtOAc (50 mL x 3) and CH₂Cl₂ (30 mL). The organic layers were washed with H₂O and brine (25 mL each). Combined organic layers were dried over MgSO₄, concentrated and purified by flash chromatography with 6:94 (10% conc NH₄OH in MeOH)-CH₂Cl₂ to afford 79.8 mg (16%) of the desired alkylated product along with 115.3 mg (37%) of the unreacted indole. The oxalate salt was formed in 96% by the method previously described.

FDMS 510 (M+1); Anal. Calcd for C₃₇H₄₃N₃O₁₀·0.9C₄H₈O₂: C, 63.41; H, 6.58; N, 5.46. Found: C, 63.64; H, 6.48; N, 5.10.

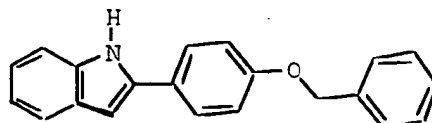
-43-

Example 8

Preparation of Methyl 4-[4-[1-[3-Methoxy-4-[(1-pyrroli-
diny]methyl]benzyl]indol-2-yl]phenoxy]butyrate Oxalate.



A. 2-(4-Benzoyloxyphenyl)indole.



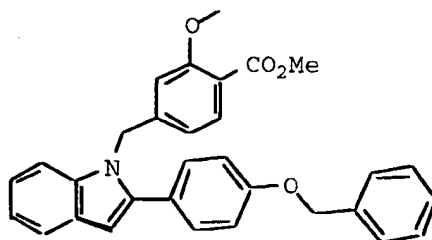
To 47.2 g of polyphosphoric acid was added 13.62 g
(0.10 mol) of 4'-hydroxyacetophenone and 10.0 mL (0.10 mol)
of phenylhydrazine at room temperature. The mixture was
heated at 150 °C (oil bath temp) for 1 h. After cooling to
room temperature an ice-cold H₂O (250 mL) was added and the
dark brown mixture was stirred overnight. This was then
neutralized with 5.0 N NaOH (100 mL) and extracted with
EtOAc (1 L x 4) which was washed with saturated aqueous
NaHCO₃ and brine (500 mL each). Organic layers were dried
over MgSO₄, filtered through a pad of diatomaceous earth,
and concentrated to ca. 200-300 mL. This was treated with
charcoal, filtered and concentrated. The crude 2-(4-
hydroxyphenyl)indole 12.02 g (57%) was obtained. The crude
phenolic indole (10.7 g; 51 mmol) was treated with 33.32 g
(0.10 mol) of Cs₂CO₃ and 6.7 mL (56 mmol) of benzyl bromide
in ca. 250 mL of DMF at room temperature overnight. The
mixture was filtered through a pad of diatomaceous earth
with thorough EtOAc rinse. The filtrate was taken up in to
1 L of EtOAc and washed with H₂O (500 mL x 2) and brine (500
mL) which were back-extracted with EtOAc (1 L x 3).

-44-

Combined organic layers were dried over MgSO_4 , concentrated and purified by PrepLC with 10:90 benzene-hexanes and 10:10:80 benzene- Et_2O -hexanes to obtain 5.34 g (27%) of N,O-dibenzylated product and 1.94 g (13%) of the named benzyl ether.

FDMS 299 (M+); Anal. Calcd for $\text{C}_{21}\text{H}_{17}\text{NO}$: C, 84.25; H, 5.72; N, 4.68. Found: C, 84.20; H, 5.71; N, 4.60.

10 **B. Methyl 3-Methoxy-4-[[2-(4-benzyloxyphenyl)indol-1-yl]-methyl]benzoate.**

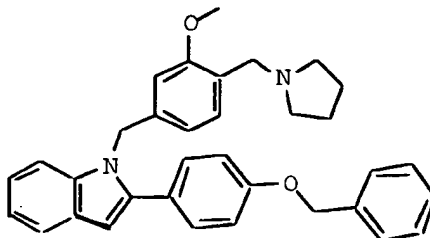


15 A mixture of 109.4 mg (2.4 mmol) of the above indole (Part A) and 147 mg of KOH in 15 mL of anhydrous DMSO was stirred at room temperature for 1 h. To this was cannulated 614 mg (2.4 mmol) of methyl 4-bromomethyl-2-methoxybenzoate in 5.0 mL of DMSO at room temperature. The mixture was
20 stirred overnight and then the reaction was quenched with 1.1 mL of 2.5 N HCl. The mixture was taken up in 250 mL of EtOAc and washed with H_2O (150 mL x 2) and 1:1 saturated aqueous NH_4Cl -brine (160 mL). The aqueous layers were back-extracted with EtOAc (250 mL x 2). Combined organic layers
25 were dried over MgSO_4 and concentrated. The residue was crystallized from EtOAc. The precipitate formed was identified as the unreacted indole after filtration and drying (163.5 mg, 23%). The filtrate was concentrated and purified by PrepLC and flash chromatography with 10~30%
30 EtOAc-hexanes to afford 280.6 mg (25%) of the title compound and 246.9 mg (16%) of the N,C₃-dialkylated product.

-45-

FDMS 477 (M⁺); Anal. Calcd for C₃₁H₂₇NO₄·0.12CH₂Cl₂: C, 76.63; H, 5.63; N, 2.87. Found: C, 76.60; H, 5.32; N, 2.60.

- 5 C. 2-(4-Benzyloxyphenyl)-1-[[3-methoxy-4-(1-pyrrolidinyl)-methyl]benzyl]indole.



- 10 The title compound was obtained in 94% yield in two steps from the methyl benzoate (Part B) via its LAH reduction to the corresponding benzylic alcohol, followed by the displacement of the hydroxy group with pyrrolidine via a mesylate formation as described above in Example 1.

15

FDMS 502 (M⁺); Anal. Calcd for C₃₄H₃₄N₂O₂·0.11CH₂Cl₂: C, 80.02; H, 6.74; N, 5.47. Found: C, 80.04; H, 6.61; N, 5.64.

- 20 D. Methyl 4-[4-[1-[3-Methoxy-4-[(1-pyrrolidinyl)methyl]benzyl]indol-2-yl]phenoxy]butyrate Oxalate.

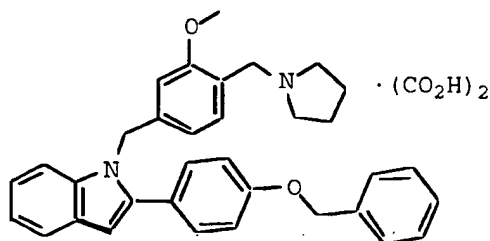
- 25 The title compound was prepared in 66% yield in three steps from the benzyl ether (Part C) via debenzylation by hydrogenolysis, alkylation of the resultant phenol with methyl 4-chlorobutyrate, and oxalate formation as described in Example 1-E.

- 30 FDMS 512.2 (M⁺); Anal. Calcd for C₃₄H₃₈N₂O₈·0.3C₄H₈O₂: C, 67.20; H, 6.47; N, 4.45. Found: C, 67.20; H, 6.73; N, 4.55.

-46-

Example 9

Preparation of 2-(4-Benzyloxyphenyl)-1-[[3-methoxy-4-(1-pyrrolidinyl)methyl]benzyl]indole Oxalate.

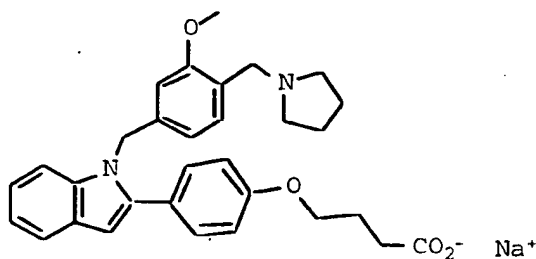


The title compound was prepared in a quantitative yield from the free base (Example 8, Part C) as described in Example 1-E.

FDMS 503 (M+1); Anal. Calcd for $\text{C}_{34}\text{H}_{34}\text{N}_2\text{O}_2 \cdot 0.9\text{C}_2\text{H}_2\text{O}_4 \cdot 0.7\text{C}_4\text{H}_8\text{O}_2$: C, 71.84; H, 6.47; N, 4.34. Found: C, 71.91; H, 6.83; N, 4.11.

Example 10

Preparation of Sodium [4-[1-[3-Methoxy-4-[(1-pyrrolidinyl)-methyl]benzyl]indol-2-yl]phenoxy]butyrate.



The title compound was obtained by hydrolysis of the methyl ester (free base of Example 8, Part D) with 1.0 N NaOH as previously described.

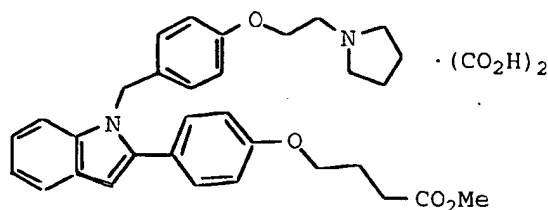
FDMS 521 (M+1), 499 (base, -Na); Anal. Calcd for $\text{C}_{31}\text{H}_{33}\text{N}_2\text{O}_4 \cdot 0.7\text{NaOH}$: C, 67.87; H, 6.19; N, 5.11. Found: C, 67.75; H, 5.98; N, 5.17.

-47-

Example 11

Preparation of Methyl 4-[4-[1-[4-[2-(1-Pyrrolidinyl)ethoxy]benzyl]indol-2-yl]phenoxy]butyrate Oxalate.

5



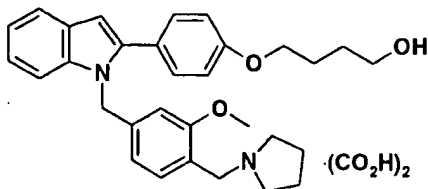
A mixture of 2-(4-benzylloxyphenyl)indole (505.0 mg, 1.7 mmol) and 0.30 mL of TlOEt in 15 mL of anhydrous benzene was heated at reflux while azeotropically removing the EtOH formed by discarding and replenishing benzene (5 mL x 3) via a Dean-Stark trap for 30 min. The mixture was heated at reflux for another 1 h and then concentrated to ca. 3-4 mL by distilling off the excess benzene. The mixture was cooled in a water bath, and 465.9 mg (1.7 mmol) of 4-[2-(1-pyrrolidinyl)ethoxy]benzyl chloride hydrochloride was added to the solution in one portion. The reaction mixture was then heated at reflux for 24 h. The reaction was quenched with saturated aqueous NaHCO₃ and extracted with CH₂Cl₂ (50 mL x 3). The organic layers were washed with 1:1 saturated aqueous NaHCO₃-brine, combined, dried over MgSO₄, and concentrated. Purification by flash chromatography three times gave not-yet-pure N-alkylated indole intermediate (90.4 mg). Debenzylation of the benzyl ether by hydrogenolysis, alkylation of the resultant phenol with methyl 4-chlorobutyrate, and oxalate salt formation as before yielded 21.2 mg (2% for 4 steps) of the title compound.

FDMS (M+1); Anal. Calcd for C₃₄H₃₈N₂O₈: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.32; H, 6.99; N, 4.26.

-48-

Example 12

Preparation of 4-[4-[1-[3-Methoxy-4-[(1-pyrrolidinyl)-methyl]benzyl]indol-2-yl]phenoxy]butanol Oxalate.



5

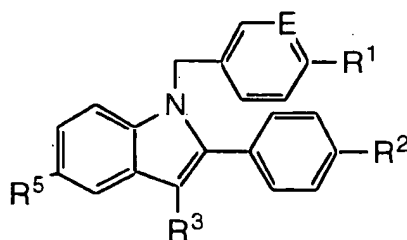
The title compound was prepared in 70% yield in two steps from LAH reduction of methyl 4-[4-[1-[3-methoxy-4-[(1-pyrrolidinyl)methyl]benzyl]indol-2-yl]phenoxy]butyrate (free base of Example 8) in THF at 0 °C for 1 h, followed by
10 oxalate formation as previously described.

mp (free base) 42-45 °C; FDMS 484.2 (M+); Anal. Calcd for C₃₃H₃₈N₂O₇·0.5C₄H₈O₂: C, 67.94; H, 6.84; N, 4.53. Found: C, 68.01; H, 7.22; N, 4.33.

-49-

What is claimed is:

1. A method of inhibiting thrombin comprising using an effective amount of a thrombin inhibiting compound of formula I (or a pharmaceutically acceptable salt thereof)



I

wherein

- E is CH or CR^e in which R^e is methyl, methoxy or halo;

- R¹ is carboxy, [(1-4C)alkoxy]carbonyl, hydroxymethyl or -X¹-(CH₂)_s-NR^sR^t in which X¹ is a direct bond, methylene or O; s is 1 or 2; provided that when s is 1, then X³ is a direct bond; and R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino;

- R² is benzyloxy, -X²-(CH₂)_m-NR^aR^b in which X² is a direct bond, methylene, O or S; m is 1, 2, 3, 4 or 5; provided that when m is 1, then X² is a direct bond; and R^a and R^b are independently hydrogen or (1-3C)alkyl or the group NR^aR^b is pyrrolidino, piperidino, or morpholino; or

- R² is -X²-(CH₂)_n-R^f in which X² is a direct bond, methylene or O; n is 1, 2 or 3; and R^f is 5-tetrazolyl, carboxy, [(1-4C)alkoxy]carbonyl or hydroxymethyl;

- R³ is hydrogen, chloro or a benzyl group which may bear a methyl or methoxy substituent at the 3-position and a [(1-4C)alkoxy]carbonyl substituent at the 4-position; and

- R⁵ is hydrogen, hydroxy or methoxy; and provided that at least one of R¹ and R² includes an amino moiety -NR^sR^t or -NR^aR^b.

-50-

2. The method of claim 1 wherein the compound of formula I is one in which

E is CH or CR^e in which R^e is methyl, methoxy or bromo;

5 R¹ is -X¹-(CH₂)_s-NR^sR^t;

R² is -X²-(CH₂)_m-NR^aR^b or is -X²-(CH₂)_n-R^f in which X² is O; n is 3; and R^f is carboxy, [(1-4C)alkoxy]-carbonyl or hydroxymethyl;

R³ is hydrogen or chloro; and

10 R⁵ is hydrogen, hydroxy or methoxy.

3. The method of claim 1 or 2 wherein the compound of formula I is one in which

E is CH or CR^e in which R^e is methoxy;

15 R¹ is -X¹-(CH₂)_s-NR^sR^t in which X¹ is a direct bond, methylene or O; s is 1 or 2; provided that when s is 1, then X³ is a direct bond; and R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino;

20 R² is -X²-(CH₂)_m-NR^aR^b in which X² is O, m is 2, and the group NR^aR^b is pyrrolidino, piperidino, or morpholino; or

R² is -X²-(CH₂)_n-R^f in which X² is O; n is 1, 2 or 3; and R^f is carboxy or [(1-4C)alkoxy]carbonyl;

25 R³ is hydrogen; and

R⁵ is hydrogen, hydroxy or methoxy.

4. The method of any one of claims 1-3 wherein halo is fluoro, chloro, bromo or iodo; a (1-3C)alkyl group is methyl, ethyl, propyl or isopropyl; and a (1-4C)alkoxy group is methoxy, ethoxy, isopropoxy or t-butoxy.

5. The method of any one of claims 1-4 in which, independently:

35 E is CH or CR^e in which R^e is methoxy;

R¹ is pyrrolidinomethyl or 2-pyrrolidinoethoxy;

-51-

R^2 is 2-pyrrolidinoethoxy or R^2 is $-X^2-(CH_2)_n-R^f$ in which X^2 is O, n is 3, and R^f is carboxy or methoxy-carbonyl;

R^3 is hydrogen; and

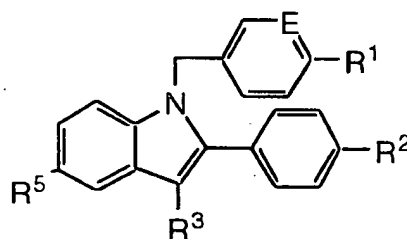
5 R^5 is hydrogen.

6. The method of claim 1 wherein said compound of formula I is 1-[3-methoxy-4-(1-pyrrolidinyl)methyl]-benzyl-2-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]indole.

10

7. A pharmaceutical formulation comprising in association with a pharmaceutically acceptable carrier, diluent or excipient, a thrombin inhibiting compound of formula I (or a pharmaceutically acceptable salt thereof)

15



I

wherein

20 E is CH or CR^e in which R^e is methyl, methoxy or halo;

R^1 is carboxy, [(1-4C)alkoxy]carbonyl, hydroxymethyl or $-X^1-(CH_2)_s-NR^sR^t$ in which X^1 is a direct bond, methylene or O; s is 1 or 2; provided that when s is 1, then X^3 is a direct bond; and R^s and R^t are independently 25 hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino;

R^2 is benzyloxy, $-X^2-(CH_2)_m-NR^aR^b$ in which X^2 is a direct bond, methylene, O or S; m is 1, 2, 3, 4 or 5; provided that when m is 1, then X^2 is a direct bond; and R^a 30 and R^b are independently hydrogen or (1-3C)alkyl or the group NR^aR^b is pyrrolidino, piperidino, or morpholino; or

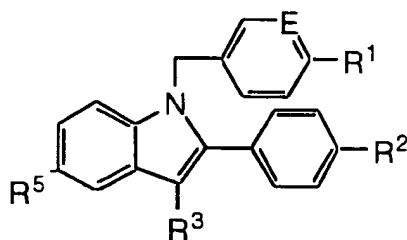
-52-

R^2 is $-X^2-(CH_2)_n-R^f$ in which X^2 is a direct bond, methylene or O; n is 1, 2 or 3; and R^f is 5-tetrazolyl, carboxy, [(1-4C)alkoxy]carbonyl or hydroxymethyl;

R^3 is hydrogen, chloro or a benzyl group which may bear a methyl or methoxy substituent at the 3-position and a [(1-4C)alkoxy]carbonyl substituent at the 4-position; and

R^5 is hydrogen, hydroxy or methoxy; and provided that at least one of R^1 and R^2 includes an amino moiety $-NR^sR^t$ or $-NR^aR^b$.

8. A compound of formula I (or a pharmaceutically acceptable salt thereof)



I

wherein

E is CH or CR^e in which R^e is methyl, methoxy or halo;

R^1 is carboxy, [(1-4C)alkoxy]carbonyl, hydroxymethyl or $-X^1-(CH_2)_s-NR^sR^t$ in which X^1 is a direct bond, methylene or O; s is 1 or 2; provided that when s is 1, then X^3 is a direct bond; and R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino;

R^2 is benzyloxy, $-X^2-(CH_2)_m-NR^aR^b$ in which X^2 is a direct bond, methylene, O or S; m is 1, 2, 3, 4 or 5; provided that when m is 1, then X^2 is a direct bond; and R^a and R^b are independently hydrogen or (1-3C)alkyl or the group NR^aR^b is pyrrolidino, piperidino, or morpholino; or

R^2 is $-X^2-(CH_2)_n-R^f$ in which X^2 is a direct bond, methylene or O; n is 1, 2 or 3; and R^f is 5-tetrazolyl, carboxy, [(1-4C)alkoxy]carbonyl or hydroxymethyl;

-53-

R^3 is hydrogen, chloro or a benzyl group which may bear a methyl or methoxy substituent at the 3-position and a [(1-4C)alkoxy]carbonyl substituent at the 4-position; and

R^5 is hydrogen, hydroxy or methoxy; and

provided that at least one of R^1 and R^2 includes an amino moiety $-NR^sR^t$ or $-NR^aR^b$; and further

provided that the compound is not one wherein E is CH or CR^e in which R^e is methyl, methoxy or halo; R^1 is $-X^1-(CH_2)_s-NR^sR^t$ in which X^1 is O; s is 2; R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino; R^2 is benzyloxy; R^3 is hydrogen or chloro; and R^5 is hydrogen, hydroxy or methoxy.

9. The compound (or salt thereof) of claim 8 wherein the compound of formula I is one in which

E is CH or CR^e in which R^e is methyl, methoxy or bromo;

R^1 is $-X^1-(CH_2)_s-NR^sR^t$;

R^2 is $-X^2-(CH_2)_m-NR^aR^b$ or is $-X^2-(CH_2)_n-R^f$ in which X^2 is O; n is 3; and R^f is carboxy, [(1-4C)alkoxy]-carbonyl or hydroxymethyl;

R^3 is hydrogen or chloro; and

R^5 is hydrogen, hydroxy or methoxy.

10. The compound (or salt thereof) of claim 8 or 9 wherein the compound of formula I is one in which

E is CH or CR^e in which R^e is methoxy;

R^1 is $-X^1-(CH_2)_s-NR^sR^t$ in which X^1 is a direct bond, methylene or O; s is 1 or 2; provided that when s is 1, then X^3 is a direct bond; and R^s and R^t are independently hydrogen or (1-3C)alkyl or the group NR^sR^t is pyrrolidino, piperidino, or morpholino;

R^2 is $-X^2-(CH_2)_m-NR^aR^b$ in which X^2 is O, m is 2, and the group NR^aR^b is pyrrolidino, piperidino, or morpholino; or

-54-

R^2 is $-X^2-(CH_2)_n-R^f$ in which X^2 is O; n is 1, 2 or 3; and R^f is carboxy or [(1-4C)alkoxy]carbonyl;

R^3 is hydrogen; and

R^5 is hydrogen, hydroxy or methoxy.

5

11. The compound (or salt thereof) of any one of claims 8-10 wherein halo is fluoro, chloro, bromo or iodo; a (1-3C)alkyl group is methyl, ethyl, propyl or isopropyl; and a (1-4C)alkoxy group is methoxy, ethoxy, isopropoxy or t-butoxy.

10

12. The compound (or salt thereof) of any one of claims 8-11 in which, independently:

E is CH or CR^e in which R^e is methoxy;

15

R^1 is pyrrolidinomethyl or 2-pyrrolidinoethoxy;

R^2 is 2-pyrrolidinoethoxy or R^2 is $-X^2-(CH_2)_n-R^f$ in which X^2 is O, n is 3, and R^f is carboxy or methoxy-carbonyl;

R^3 is hydrogen; and

20

R^5 is hydrogen.

13. The compound (or salt thereof) of claim 8 wherein said compound of formula I is 1-[3-methoxy-4-(1-pyrrolidinyl)methyl]benzyl-2-[4-[2-(1-pyrrolidinyl)-ethoxy]phenyl]indole.

25

14. A pharmaceutically acceptable salt of a novel compound of formula I as claimed in any one of claims 8-13 which is an acid-addition salt made with an acid which provides a pharmaceutically acceptable anion or a salt made with a base which provides a pharmaceutically acceptable cation.

30

15. A pharmaceutical formulation comprising in association with a pharmaceutically acceptable carrier, diluent or excipient, a novel compound of formula I (or a

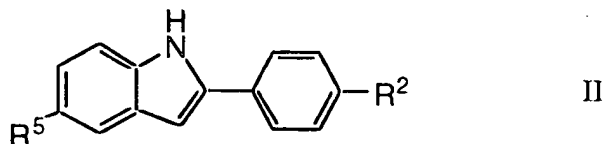
35

-55-

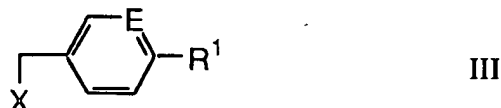
pharmaceutically acceptable salt thereof) as claimed in any of claims 8-14.

16. A process for preparing a novel compound of formula I (or a pharmaceutically acceptable salt thereof) as claimed in any of claims 8-14 which is selected from:

(a) for a compound of formula I in which R^3 is hydrogen, alkylating a compound of formula II



with an alkylating agent of formula III



in which X is a conventional leaving group, using a standard indole alkylation procedure;

(b) for a compound of formula I in which R^1 or R^f is hydroxymethyl, reducing a corresponding compound of formula I in which R^1 or R^f is [(1-4C)alkoxy]carbonyl;

(c) for a compound of formula I in which R^1 or R^2 is $-X^1-(CH_2)_s-NR^sR^t$ or $-X^2-(CH_2)_m-NR^aR^b$, respectively, alkylating an amine of formula $H-NR^sR^t$ or $H-NR^aR^b$, respectively, with a compound corresponding to formula I, but in which R^1 or R^2 is $-X^1-(CH_2)_s-X$ or $-X^2-(CH_2)_m-X$, respectively, in which X is a conventional leaving group, using a standard alkylation procedure;

(d) for a compound of formula I in which R^3 is chloro, chlorinating a corresponding compound of formula I in which R^3 is hydrogen using a conventional procedure;

(e) for a compound of formula I in which X^1 or X^2 is O, alkylating a phenolic compound corresponding to formula I, but in which R^1 or R^2 , respectively, is hydroxy using a

-56-

reagent of formula $X-(CH_2)_s-NR^sR^t$ or of formula $X-(CH_2)_m-NR^aR^b$ or $X-(CH_2)_n-R^f$, respectively, in which X is a conventional leaving group, using a standard alkylation procedure;

5 (f) for a compound of formula I in which R^1 or R^f is carboxy, decomposing the ester of a corresponding compound of formula I in which R^1 or R^f is [(1-4C)alkoxy]carbonyl;

(g) for a compound of formula I in which R^3 is a benzyl group which may bear a methyl or methoxy substituent
10 at the 3-position and a [(1-4C)alkoxy]carbonyl substituent at the 4-position, alkylating a corresponding compound of formula I in which R^3 is hydrogen using a corresponding benzyl reagent bearing a leaving group X at the α -position using a conventional procedure;

15 whereafter, for any of the above procedures, when a functional group is protected using a protecting group, removing the protecting group;

whereafter, for any of the above procedures, when a pharmaceutically acceptable salt of a compound of formula I
20 is required, it is obtained by reacting the basic form of such a compound of formula I with an acid affording a physiologically acceptable counterion or by reacting the acidic form of such a compound of formula I with an base affording a physiologically acceptable counterion or by any
25 other conventional procedure; and

wherein, unless otherwise described, E, R^1 , R^2 , R^3 and R^5 have the values described in any of claims 8-14.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/08698

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/231.8, 232.5, 316, 319, 414, 415; 544/127, 129; 546/187, 201; 548/467, 508

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96/03375 A1 (SCHERING AKTIENGESELLSCHAFT) 08 February 1996, pages 1-4.	1-3, 6-10, 13
A, P	EP 0 802 183 A1 (AMERICAN HOME PRODUCTS CORP.) 22 October 1997, pages 1-18.	1-3, 6-10, 13
A	US 5,576,343 A (NAGAHARA et al.) 19 November 1996, columns 2-8, 106.	1-3, 6-10, 13



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

&

document member of the same patent family

Date of the actual completion of the international search

17 JUNE 1998

Date of mailing of the international search report

19 AUG 1998

Name and mailing address of the ISA-US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

Deborah Lawrence For
EVELYN HUANG

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/08698

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-5, 11-12, 14-16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/08698

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 31/40, 31/445, 31/535; C07D 209/12, 209/14, 401/10, 401/14, 413/10, 413/14

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/231.8, 232.5, 316, 319, 414, 415; 544/127, 129; 546/187, 201; 548/467, 508